# United States Department of Agriculture
# Agricultural Marketing Service, Science & Technology
# Microbiological Data Program

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| SOP No: MDP-MTH-03A | | Page 1 of 7 |
| Title: Isolation and Identification of *Salmonella* from Fresh Produce | | |
| Revision: Original | Replaces: NA | Effective: 02/01/05 |

## 1. Purpose

To provide a standard procedure for the isolation and identification of *Salmonella* from fresh produce for all laboratories participating in the Microbiological Data Program (MDP).

## 2. Scope

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities. This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall have written procedures that provide specific details concerning how the procedure has been implemented in that laboratory.

## 3. Principle

Salmonella is isolated on selective media and identified by biochemical tests including automated systems such as VITEK® developed by bioMérieux. The reliability and accuracy are a result of the use of a panel of biochemical tests that are used to characterize the test organism. The resulting profile is compared to known profiles of numerous microorganisms and a subsequent identification is made.

## 4. Outline of Procedures

## 5. References:

5.1. VITEK® Users Manual

5.2. Andrews WH, and Hammack TS. *Salmonella* (Chapter 5). BAM online. FDA. April 2003. http://www.cfsan.fda.gov/~ebam/bam-5.html (last accessed 11-11-04)

5.3. June, G.A., Sherrod, P.S., Hammack, T.S.; Amaguana, R.M., and Andrews, W.H. 1995.Relative effectiveness of selenite cystine broth, tetrathionate broth, and Rappaport-Vassiliadis medium for the recovery of *Salmonella* from raw flesh and other highly contaminated foods: Precollaborative study. J. AOAC Int. 78:375- 380.

5.4. Maijala R, Johansson T, and Hirn J. 1992. Growth of *Salmonella* and competing flora in five commercial RV media. International Journal of Food Microbiology. Vol.17, pp.1-8.

5.5. SOP MDP-MTH-04, Detection of Salmonella in Fresh Produce by BAX PCR

5.6. SOP MDP-DATA-01, Record Keeping and Results Reporting

5.7. SOP MDP-MTH-03, *Salmonella* Cultural Method

5.8. SOP MDP-SHIP-03, Procedures for Packaging, Shipping, and Archiving Microbiological Cultures

5.9. SOP MDP-QA-03, Quality Assurance (QA) Controls

## 6. Specific Procedures

6.1. Equipment and Materials

6.1.1. VITEK® System

6.1.2. VITEK® assay cards: GNI+ Card (Gram-Negative Plus) V1316

6.1.3. Water bath, capable of 42 ± 0.2°C

6.1.4. Additional equipment as referenced in a participating laboratory's internal procedures

6.2. Media and Reagents

6.2.1. Rappaport-Vassiliadis (RV) medium - 16 x 150 mm sterile test tubes containing 10 mL aliquots.

NOTE: If using commercial media, Oxoid brand of RV broth is preferred.

6.2.2. Tetrathionate (TT) broth - 16 x 150 mm sterile test tubes containing 10 mL aliquots. On the day of use, add 20 mL iodine solution and 10 mL of sterile 0.1% aqueous Brilliant Green solution per 1 liter basal broth. To prepare the iodine solution, dissolve 5 g potassium iodide in 5 mL sterile distilled water, add 6 g resublimed iodine and stir to dissolve. Dilute to 20 mL.

6.2.3. Xylose lysine deoxycholate agar (XLD)

6.2.4. Hektoen enteric agar (HE)

6.2.5. Bismuth sulfite agar (BS), use 24-48 h after preparation.

6.2.6. Triple Sugar Iron agar (TSI)

6.2.7. Lysine Iron Agar (LIA)

6.2.8. Blood Agar

6.2.9. Additional media as referenced in a participating laboratory's internal procedures.

5.1.1. Polyvalent somatic O and flagellar H antisera for *Salmonella*

6.3. List of Controls (Specific strains are listed in SOP MDP-QA-03)

6.3.1. Carry all controls from all screening methods previously completed through this entire procedure. See SOPs MDP-MTH-04 and MDP-MTH-02.

6.3.2. If any of the controls fail to yield a satisfactory result refer to SOP MDP-QA-03.

6.4. Isolation of *Salmonella*

6.4.1. Selective Enrichment (only for samples screened by BAX)

6.4.1.1. Transfer 1 mL of the lactose culture (see SOP MDP-MTH-04) from each of the three individual samples that comprised the BAX® positive sample and all controls into 10 mL TT broth and incubate at 42 ± 0.2°C for 18-24 h.

6.4.1.2. Transfer 0.1 mL of lactose culture from all controls and each of the three individual samples that comprised the pooled BAX® positive sample into 10 mL of RV broth and incubate for 18-24 h at 42 ± 0.2°C.

6.4.2. Selective Plating

6.4.2.1. Streak approximately 10 µL of incubated RV broth on selective and differential plates of BS, HE, and XLD for colony isolation. Repeat streaking with TT broth on plates of BS, HE, and XLD.

NOTE: For samples screened by SOP MDP-MTH-02, also streak both M-broths.

6.4.2.2. Incubate BS, HE, and XLD for 18-24 h at 35 ± 1°C. (BS should be reincubated an additional 24 h at 35 ± 1ºC).

6.4.2.3. After 18-24 h incubation, examine BS, HE, and XLD for presence of typical and atypical colonies that may be *Salmonella*. BS should also be reexamined after 42-48 h for *Salmonella* growth.

6.4.3. Isolation

6.4.3.1. Select one or more typical colonies from one or more selective agar plates; inoculate TSI and LIA and incubate 35 ± 1ºC for 18-24 h. Streak suspect TSI or LIA to blood agar plates.

6.5. VITEK® Identification

6.5.1.1. Run VITEK® according to manufacturer's instructions. If VITEK® analysis fails to identify isolate as *Salmonella*, further evaluation is necessary. Probabilities of < 90% on VITEK® may indicate mixed or rough cultures. Re-streaking or picking additional colonies may be necessary.

NOTE: If VITEK® system is unavailable, perform an alternative official standard method of identification.

6.6. Serology

6.6.1. Perform serology per participant's internal laboratory procedures for polyvalent somatic O and flagellar H antigens.

6.7. Reporting

6.7.1. Immediately following completion of biochemical and serological tests submit (e.g., fax or e-mail) updated "Preliminary/Final Results Notification Form" per SOP MDP-DATA-01.

6.7.2. Report results according to SOP MDP-DATA-01.

6.8. Transport and Storage of Cultures

6.8.1. Refer to SOP MTH-SHIP-03 for shipping procedures.

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MDP-MTH-03A Original January 2005 Monitoring Programs Office

- Change from MDP-MTH-03, Revision 02 to MDP-MTH-03A
- Removed specific details concerning FDA cultural confirmation methodology
- Added control requirements
- Removed references to specific strains
- Defined VITEK® positive results

MDP-MTH-03 Revision 02 December 2003 Monitoring Programs Office

- Added procedures to isolate *Salmonella* culturally from the sample

MDP-MTH-03 Revision 01 May 2003 Monitoring Programs Office

- Updated references
- Removed instructions on operating instrument
- Changed from selenite cystine (SC) broth to Rappaport-Vassiliadis (RV) medium; added recommendation for use of Oxoid RV Broth
- Removed reference to SOP MDP-QA-01